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Genetic and Molecular Analysis of Six Tumor Suppressor Genes in *Drosophila melanogaster*

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Six *Drosophila melanogaster* tumor suppressor genes causing malignant or benign tumors in specific cell types are described. The wild-type alleles of these genes are instrumental in the differentiation of particular cell types. In the homozygous state, recessive mutations in the genes interrupt the differentiation of the cells and thus cause their uncontrolled, autonomous, lethal proliferation. The tumors show all major characteristics of malignant and benign neoplastic growth. Genomic sequences of four of the genes have been identified and are currently being characterized.

Introduction

The body plan of Drosophila is determined by a series of maternal and zygotic genes (*1,2*). The developmental and molecular analysis of these genes is unfolding a complex network of interactions. Simultaneously with the determination of the body pattern, other genes become active that determine the developmental fate of the larval and adult tissues. The subject of this paper is six tumor suppressor genes involved in the determination of specific presumptive adult cell types in the embryo and their malfunction during development. In the homozygous mutant state, these genes cause differentiation arrest and malignant and/or benign neoplastic transformation of cell types, such as the adult optic neuroblasts in the larval brain, the imaginal discs, the larval blood cells, and the gonial cells (*3–5*). We are currently involved in the molecular cloning of six tumor-suppressor genes (Table 1).

Table 1. Designations, genetic loci, cytogenetic loci, and number of alleles in six tumor-suppressor genes of *Drosophila melanogaster*.

Designation of gene	Genetic locus, cM	Cytogenetic locus	Number of mutant alleles
l(3)mbt	3– 93	97F11	3
l(3)mbn	3– 13.3 ± 0.3	64F4-5	24
l(1)mbn	1– 27.6 ± 0.2	8D8-9	2
b(2)gcn	2–106.7	60A3-7	11
l(1)mbn	1– 34.4	10B5-17	22
l(2)tud	2–104 ± 1	59F5-8	4

Lethal (3) Malignant Brain Tumor

One of the three *l(3)mbt* alleles is temperature sensitive (*4*). At the restrictive temperature of 29°C, all animals develop a brain tumor (Fig. 1*a*) and die around the time of puparium formation. Histological sections through the mutant brain reveal an invasive tumor consisting of adult optic neuroblasts and optic ganglion mother cells. Shift up and shift down experiments show a temperature-sensitive phase for tumor development spanning the embryonic, first, and second larval stages. During this time, brain tumor development is reversible upon shifting the animals down to 22°C. During the third larval instar, however, the brain tumor becomes irreversibly established.

Shift up experiments after the temperature-sensitive phase for tumorgenicity and throughout adult development causes female sterility. The defects in oogenesis are highly specific and correlate with the developmental time of the shift to the restrictive temperature (Fig. 1*b*). In

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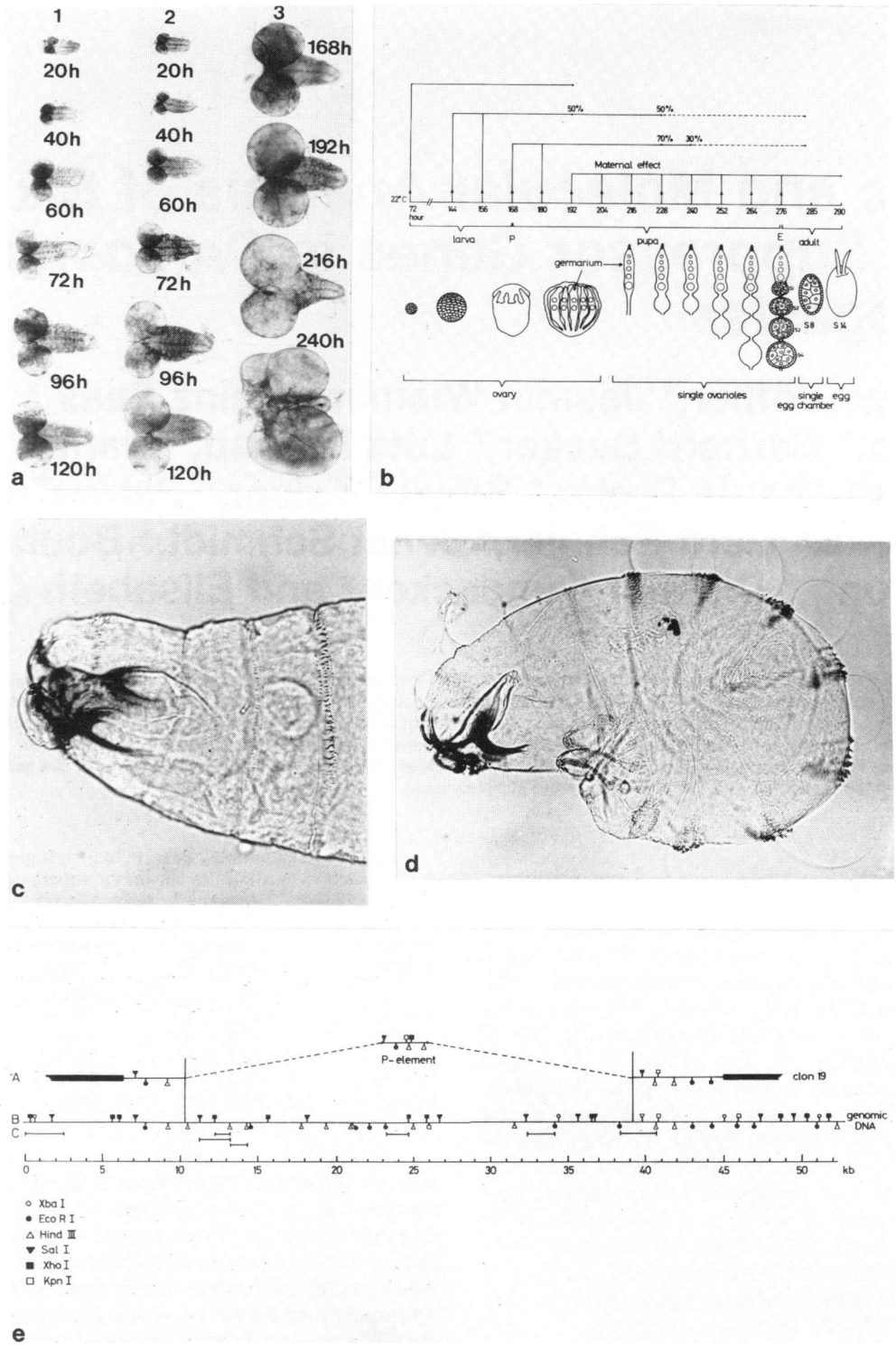

FIGURE 1. Phenotype and molecular cloning of the *l(3)mbt* brain tumor mutant. (*a*) Wild-type (row 1) and *l(3)mbt* brain-ventral-ganglion complexes (rows 2, 3) at different developmental stages in hours after egg laying. (*b*) Shift up experiments during the third larval instar and adult development, showing the normal ovarian development (lower panel) and the mutant defects (upper panel). (*c*) Larva with head defect. (*d*) Larva with dorsalized phenotype. (*e*) Restriction map of the cloned 53 kb *l(3)mbt* region (*B*), with the original clone (*A*), and 7 cDNA clones (*C*).

the earliest shift up series, oogenesis is arrested at the stem cell stage. The germaria of later shift up series exhibit fused egg chambers, but no further development is observed. Ovaries with differentiated germaria at the time of the shift up form individualized egg chambers, but the eggs are short and incapable of further development. About 25% of the deposited eggs from the late shift up series develop into segmented embryos, most of which exhibit head defects (Fig. 1*c*). More than half of these embryos show in addition to head defects thoracic and abdominal segment abnormalities. The most severe abnormality is seen in embryos that are lacking dorsal structures (Fig. 1*d*). Thus, the *l(3)mbt* wild-type allele seems to be involved in the differentiation of the adult optic neurons, the female germ line, and the body plan. In the mutant state, however, a tumor is induced only in the brain, while the germ line and tissues in the embryo do not become neoplastic.

The cloning of $l(3)mbt^{+}$ genomic sequences was accomplished by transposon tagging. A clone was isolated from a library derived from the stock containing a P-element insertion in the *l(3)mbt* gene (*6*) (Fig. 1*e*). A genomic walk in a wild-type library, encompassing 53 kb, revealed a 30 kb deletion associated with the P-element insertion (*7*) (Fig. 1*e*). Within the cloned DNA, a number of cDNAs were identified (Fig. 1*e*). Because of the weak mutant phenotype of the P-element-induced allele $l(3)mbt^{P3}$, we assume that the gene is associated with one of the two deletion breakpoints. To prove that the *l(3)mbt* gene has been cloned, we are currently performing the P-element-mediated germ line transformation with the goal of preventing development of the tumor in the *l(3)mbt* animals.

Lethal (3) Malignant Blood Cell Neoplasm

Five independent tumor suppressor genes cause malignant transformation of the larval phagocytic blood cell-type, the plasmatocytes (*4*). The tumors originate in the hematopoietic organs located along the heart. In the *l(3)mbn* mutant, the hematopoietic organs are 5 to 10 times larger than their wild-type counterparts, and in the hemolymph numerous immature plasmatocytes are present. The mutant plasmatocytes, incapable of distinguishing self from non-self invade and destroy the tissues of the larva, which dies at the time of puparium formation.

In order to clone the *l(3)mbn* gene, we determined the cytogenetic locus, generated 24 alleles (Table 1), and obtained an entry probe from a minilibrary via microcloning DNA from the giant chromosome bands 64 to 65. Three of the 144 clones from the minilibrary were used to initiate chromosomal walks. After a walk of 25 kb, we reached the breakpoint in 64F4 of a telomeric inversion located in the *l(3)mbn* gene via clone *L-6714* (Fig. 2*A*). Southern blot of the DNAs from 19 *l(3)mbn* alleles, hybridized with the *L-6714* clone, showed in all instances genomic aberrations, which indicates strongly that *l(3)mbn* gene sequences have been cloned (Fig. 2*B*).

Lethal (1) Malignant Blood Neoplasm

The *l(1)mbn* gene is the second blood tumor gene that we are attempting to clone (Table 1). The availability of a probe hybridizing to *8D4-8* (*8*) and the estimation of the DNA contents of the region to less than 250 kb, prompted the decision to initiate a genomic walk. So far about 50 kb have been walked (*9*). We are planning to probe the DNA of the two *l(1)mbn* alleles and the wild-type in Southern blots with DNA fragments from the walk.

Benign (2) Gonial Cell Neoplasm

In the *b(2)gcn* mutant, oogenesis and spermiogenesis are arrested at the stem-cell stage, which results in sterility in both sexes (*4*). The oogonialike and spermatogonialike cells remain undifferentiated and grow like a benign tumor.

Eleven *b(2)gcn* alleles were generated, one of which, a small deletion *Df(2R)106*, allowed the determination of the cytogenetic locus (Table 1). A genomic walk, initiated with a P-element probe closely linked to the *b(2)gcn* gene, spans by now about 100 kb (Fig. 3). The genomic walk in the distal direction includes the distal deletion breakpoint of *Df(2R)106* and extends into a duplication of approximately 20 kb (Fig. 3). The breakpoint of the deletion is associated with a transcriptionally active region, as a number of cDNAs were found that hybridize with the 4.8, 5.5, and 2.8 kb fragments from the breakpoint region (Fig. 3). Transformation studies are currently being initiated to test for the possible presence of the *b(2)gcn* gene in the distal breakpoint of the deletion. The centromeric deletion breakpoint has not yet been reached (Fig. 3).

Lethal (2) Tumorous Discs

The *l(2)tud* imaginal discs are characterized as tumorous by morphological criteria and by the autonomous tumorous growth *in situ* as well as after transplantation into wild-type female flies (*3,4*) (Table 1). We isolated the deficiency *Df(2R)b23* that does not contain the *l(2)tud* gene, but deletes bands 60A1-3 and, thus, brings region 59F5-8, where the *l(2)tud* gene has been localized, into juxtaposition to region 60A3,4, where the proximal breakpoint of *Df(2R)106* is located (Fig. 3). With the help of the most proximal clone from chromosomal walk 1a (*9*) (Fig. 3), we have identified three overlapping clones extending the chromosomal walk 1a into the centromeric direction with 30 kb (Fig. 3). The clone from the last chromosomal walkstep contains the distal breakpoint of deletion *Df(2R)B23* and thus makes possible the continuation of the chromosomal walk into region 59F, where *l(2)tud* is located. The four *l(2)tud* alleles will be used to determine the molecular position of the gene in Southern blots.

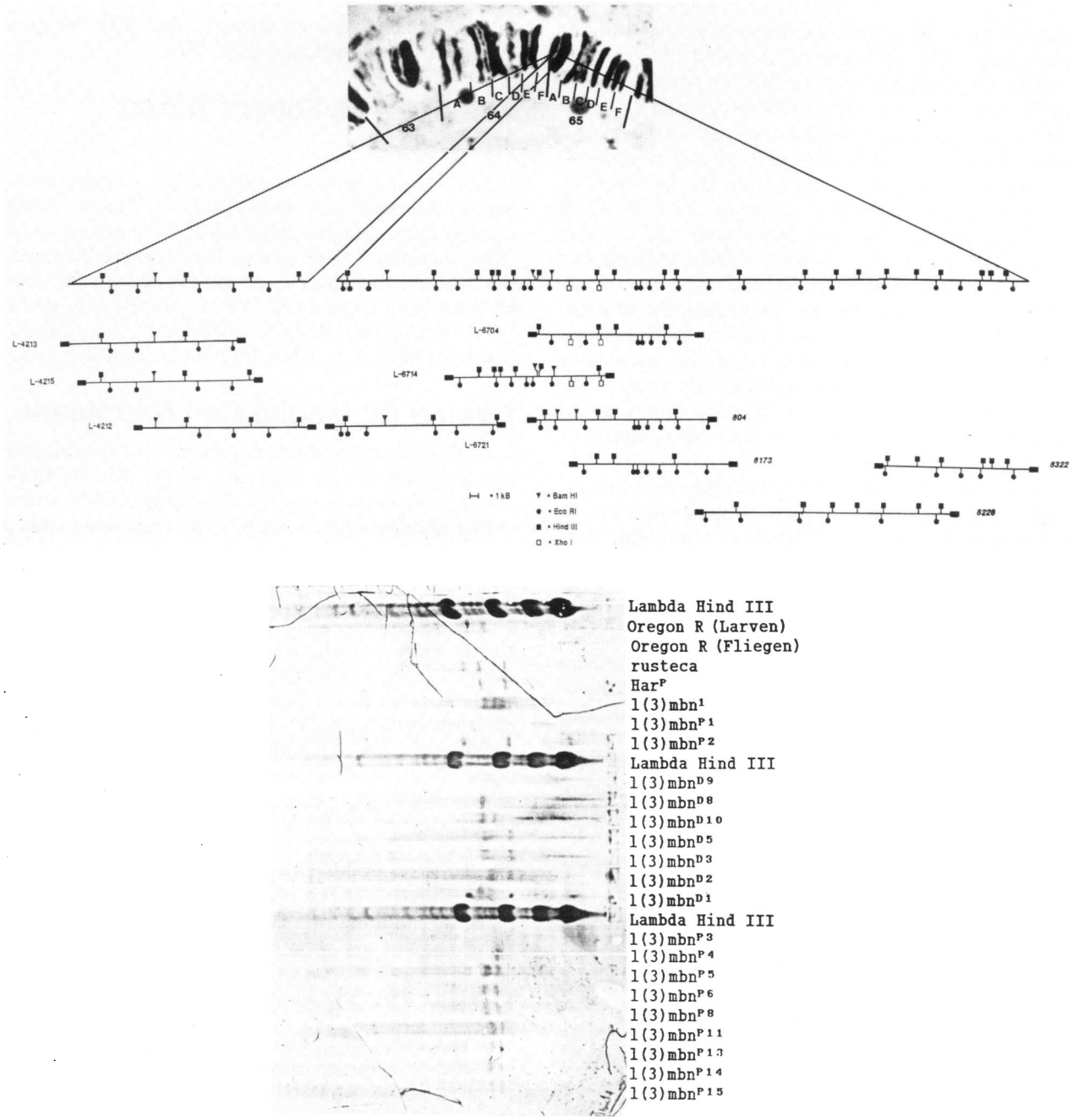

FIGURE 2. Molecular cloning of the blood tumor mutant *l(3)mbn*. (*A*) Restriction map of the cloned region in 64F on the giant chromosome together with the clones from three genomic walks. (*B*) Southern blot analysis of the DNAs from 19 *l(3)mbn* alleles and the wild-type probed with the clone *L-6714* showing variations in the banding patterns.

Lethal (1) Disc Large-1

The most prominent phenotypic aberrations in the *l(1)d.lg-1* mutant are the tumorous imaginal discs and the highly enlarged brain hemispheres (*3,10*) (Table 1). The *l(1)d.lg-1* gene region was reached via a genomic walk (*11,12*). After a detailed analysis of the genomic region, a P-element-mediated germ-line transformation will be initiated.

Discussion

In Drosophila, the etiology of malignant and benign neoplasia due to recessive mutations in specific genes is firmly established (*3,4*). The wild-type alleles of these genes seem to be causally involved in the ability of the respective cells to differentiate. In the inactivated state, differentiation does not take place, and the cells express their primordial ability to grow autonomously and in-

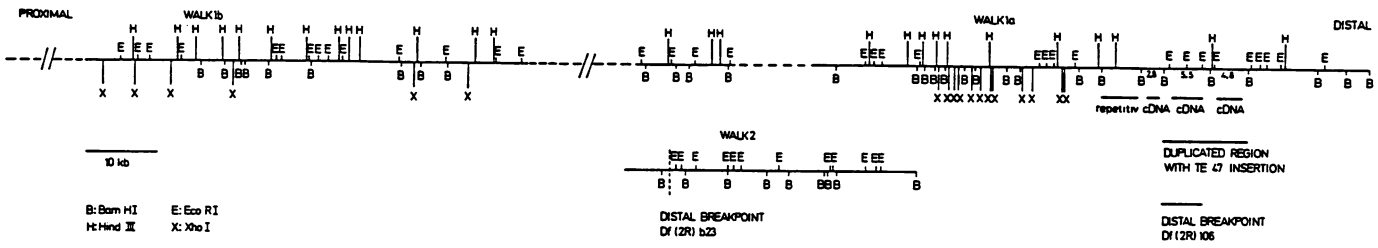

FIGURE 3. Restriction maps of three genomic walks in the deficiency *Df(2R)106* encompassing the giant chromosome bands 60A3–7. The walks were performed with the aim of cloning the *b(2)gcn* (walks 1a, 1b) and *l(2)tud* genes. Walk 1a contains the distal break point of *Df(2R)106* (arrow). Walk 1b in the proximal deletion region has not yet reached the *Df(2R)106* break point. The duplicated region and the distal break point of *Df(2R)106* are indicated. Walk 2 extends into the *Df(2R)b23*, which deletes bands 1–3 from the 60A region and thus brings the giant chromosome 60A into the vicinity of region 59F.

definitely *in situ* as well as after transplantation into wild-type hosts. The temperature-sensitive allele of the brain tumor mutant *l(3)mbt* is in this respect especially instructive. Shift up experiments show two long temperature-sensitive phases of gene activity. The early phase encompasses the embryonic, the first, and second larval stages, and the late period takes place throughout adult development. During the early period the gene is active in the adult optic neuroblasts and ganglion mother cells, while during the late phase it is expressed in the female germ line. The inactivation of the gene at the restrictive temperature of 29°C leads to different defects in the two target cells. The lack of gene function in the neuroblasts and ganglion mother cells inhibits their differentiation and causes their malignant transformation. Germ-line differentiation is also affected. However, in the germ line, the inactivation of the *l(3)mbt* gene does not induce tumorous growth but a series of stage-specific defects during oogenesis and embryonic development.

Since the differentiation of both neurons and eggs is a continuous process, taking place over a long period of time, the *l(3)mbt* gene must be interacting with a broad spectrum of cellular events in a strictly controlled temporal fashion. We assume that the remaining genes will also show a complex pattern of interactions in differentiation. Our results, together with the results on the tumor suppressor gene lethal (2) giant larvae (*13*), show that the development of these tumors is closely related to the inability of the primordial cells to differentiate.

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